様式８

論　文　内　容　要　旨

報告番号	甲　先　第　295　号	氏　名	桑江　忍
学位論文題目	Studies on chemically defined platform media for CHO cell fed-batch culture process （CHO細胞フェッドバッチ培養プロセスのためのプラットフォーム合成培地に関する研究）		

内容要旨

Monoclonal antibodies (mAbs) and Fc fusion proteins have become major drug modalities for the treatment of a wide range of diseases, especially in the areas of autoimmune/inflammatory disorders and oncology. In 2016, six of top 10 global prescription drugs were mAbs and Fc fusion proteins. To meet the demands of clinical development and the commercial market a large amount of mAb must be produced, because mAb therapies usually require large doses over a long period of time. For example, to enable phase 1 clinical trial, hundreds gram to 1 kg of purified mAb is generally required for manufacturing process development and supply of investigational medicinal product. In addition, because of the increasing number of therapeutic mAb candidates and to shorten the period of time needed to reach Phase 1 clinical trials, faster development of a high-yield cell culture process has been an area of focus among biopharmaceutical companies. The platform approach is a practical solution to enable faster development of cell culture processes. More specifically, a high yield platform fed-batch culture process that can be utilized for multiple mAb producing cell lines will eliminate a six to twelve month cell culture process development period, multiple production batches and then will finally enable faster Phase 1 clinical trial.

In this study, a chemically defined platform basal medium and feed media were developed using a single Chinese hamster ovary (CHO) cell line that produces a monoclonal antibody (mAb). Cell line A, which showed a peak viable cell density of 5.9×10^6 cells/mL and a final mAb titer of 0.5 g/L in batch culture, was selected for the platform media development. Stoichiometrically balanced feed media were developed using glucose as an indicator of cell metabolism to determine the feed rates of all other nutrients. A fed-batch culture of cell line A using the platform fed-batch medium yielded a 5 g/L mAb titer, which was 10-fold higher than that of the batch culture.

To further improve the basal medium and feed media, a metabolome analysis was performed on the cell lysate and the spent media of the fed-batch culture. Among the metabolites analyzed, choline was found to be one of the potential limiting nutrients in the fed-batch culture. To determine whether choline was a limiting nutrient in the fed-batch culture, 2-fold and 4-fold choline-enriched feed media were prepared and evaluated in fed-batch cultures using cell line A. Then the author found that choline limitation in the fed-batch culture with the original 1-fold choline feed media caused a lower cell viability, a lower mAb titer, a higher mAb aggregate content, and a higher mannose-5 content. Both the 2-fold and 4-fold choline enriched culture resulted in a 16% increase in the mAb titer, 6.4 g/L, and also resulted in the similar mAb quality improvement compared with the 1-fold choline culture. Therefore it was concluded that choline chloride to glucose ratios (g-choline chloride/g-D-glucose) of 0.0057 and 0.0114 were optimal in terms of the mAb production titer and the mAb quality for the fed-batch culture of cell line A.

To examine the applicability of the platform basal medium and feed media, three other cell lines (A16, B, and C) that produce mAbs were cultured using the platform fed-batch medium, and they yielded mAb titers of 8.4, 3.3, and 6.2 g/L, respectively. The peak viable cell densities of the three cell lines ranged from 1.3×10^7 to 1.8×10^7 cells/mL. These results show that the nutritionally balanced fed-batch medium and feeds worked well for other cell lines.

The platform basal medium and feed media developed in this study will shorten the media-development time for mAb-producing CHO cell lines and then enable faster phase 1 clinical trials.